I. Select and Prepare a Template Structure

What you need to begin:

1. Cn3D file containing the structural alignment including your query protein

Procedure:

If your alignment includes only one structure, the template is this structure.
If your alignment includes more than one structure, find the structure with the greatest sequence similarity to your query:

In Cn3D Sequence window

1. Edit/Enable editor should be checked.
2. Choose Edit/Sort rows…/Proximity Sort
3. Click anywhere on your query sequence
4. The rows (except the master structure) are now sorted by their sequence similarity relative to your query. Mouse over any sequence to view its score. Choose as the template the structure with the highest score. Be sure to check the master, as it will not move after the sort and may be the highest scoring structure!
5. Place your mouse over any aligned (capitalized) residue in the template and write down the *loc* and *PDB* residue numbers shown in the lower left. For example, if you see "loc 54 (PDB 128)" write down loc=54, pdb=128.
6. Write down the Cn3D label for both your query and template structure. The label is the text in the column to the left of the sequence. If this label begins with "gi", omit these letters and only write the number (the GI number).
7. Choose View/Export…/A2M FASTA, and enter an output filename.

II. Generate Chimera Scripts to Create an Initial Model

What you need to begin:

1. filename of A2M FASTA file of your alignment
2. label of your template structure (eg. 1C1Y_A)
3. label of your query sequence (eq. *query* or *3874524*)
4. loc and PDB residue numbers for a residue in the template structure

Procedure:

1. Open the Chimera Scripts tool in your web browser (http://www.ncbi.nlm.nih.gov/Class/Structure/Modeling/chimera_scripts.cgi)
2. Fill out the form with the required data.
3. Click on the appropriate buttons to download the three Chimera scripts and one mask file:
    a. Swap script: swap.cmd (changes residues to match query)
    b. Color script: color.cmd (colors residues as described below)
    c. Select script: select.cmd (selects the aligned region)
    d. SCWRL3.0 mask file: scmask (use with –s option)
4. Move each downloaded script into your working directory and rename them as you like.

III. Create the Initial Model Structure

What you need to begin:

1. Chimera scripts and scmask files produced in II.
2. PDB code of the structure template

Procedure:

1. Launch Chimera
2. Choose File/Fetch by ID…
3. Select PDB and enter the 4-digit PDB code of the template
4. Run the Chimera swap script: Choose File/Open and select the swap script
5. Run the Chimera color script. The colors have the following meanings
    a. White = aligned; same residue
    b. Red = aligned; different residue
    c. Blue = residue in template (structure) but NOT in query (insertion)
    d. Green = aligned; same residue; boundary of a region in query but NOT in template (deletion)
    e. Yellow = same as Green except boundary residue is different
    f. Gray = not aligned
6. Select the chain being modeled: Choose Select/Chain/*chain_id* (where *chain_id* is the one letter chain of the template)
7. Choose File/Save PDB…; click "Save selected atoms only"
8. Choose File/Close session

IV. Check Model Structure for Steric Clashes

What you need to begin:

1. PDB file of model structure produced by Chimera

Procedure:

1. Load the model structure: Choose File/Open
2. Run the Chimera color script (File/Open)
3. Select the entire chain: Select/Chain/*chain_id*
4. Choose Tools/Structure analysis/Find Clashes
    a. Click "Designate" button to activate selected atoms
    b. Select Check designated atoms against "themselves"
    c. Under Treatment of Clashes/Contacts, check (if necessary) "Select", "Color"
    d. Click Apply to run the analysis
5. Look for green circles around atoms involved in clashes. Make a note of the residues with the most problems. You can view this list by opening the Reply Log (Favorites/Reply Log)
6. Optimize side chain rotamers with SCWRL3:
    a. /scwrl3.exe –i *input_pdb* –o *output_pdb* –s *scmask*
7. Open the new PDB file. The two models will superimpose everywhere except where SCWRL3 altered side chain torsions.
8. Select the chain of the revised model (#1) as in step 3
9. Check for clashes in this model as in step 4
10. Close the session: Choose File/Close session

V. Minimize Model Structure

What you need to begin:

1. PDB file of model structure with optimized side chains

Procedure:

1. Open the model structure (File/Open)
2. Highlight clashes in the structure as in steps 3 and 4 above.
3. Run the Chimera color script (File/Open)
4. Run the Chimera select script (File/Open)
5. Choose Tools/Structure editing/Minimize structure
    a. Keep the default number of steps (100)
    b. For Fixed Atoms, choose Unselected; click Minimize
    c. In Dock Prep, accept the defaults; click OK
    d. In Add hydrogens, accept defaults; click OK; hydrogens should appear
    e. If Specify formal charges appears, accept defaults; click OK
6. Monitor the minimizer in the lower status line of the Chimera structure window, and view the results in the Reply Log (Favorites/Reply Log)
7. Repeat the Find Clashes analysis in step 2. You should find either no clashes or a very small number of clashes. If there are clashes remaining, you can run the Minimizer again with another 100 steps.
8. Save the minimized structure as a new PDB file.

VI. Import the Model Structure into Cn3D

What you need to begin:

1. Cn3D file containing the structural alignment including your query protein
2. PDB file of minimized model structure (from Chimera)

Procedure:

1. Go to the NCBI VAST Search page (Home Page/Structure/VAST Search)
2. Enter the filename of the PDB file containing the minimized model structure and click Submit.
3. When the conversion is finished, change the Display menu to Save File and click View 3D Structure.
4. Move the saved file (VS.c3d) to your project directory and rename it if you want.
5. Launch Cn3D and open the structure alignment including your query
6. In the sequence window, choose Edit/Enable Editor, then Imports/Show Imports
7. In the imports window, choose Edit/Import Structure, then choose From File
8. Align the sequence with the Block Aligner and Merge to Neighbor
9. Save the new alignment as a new Cn3D file, and then reopen this new file. Your model structure should now be visible in Cn3D.